Puri, I. M. 1924. Studies on the anatomy of *Cimex lectularius*. II. The stink organs. Ibid. **16**: 268–278.

Roth, L. M. 1961. A study of the odoriferous glands of *Scaptocoris divergens* (Hemiptera-Cydnidae). Ann. Ent. Soc. Amer. **54**(6): 900–911.

Waterhouse, D. F., D. A. Forss and R. H. Hachman. 1961. Characteristic odour components of the scent of stink bugs. J. Ins. Physiol. **6**(2): 113–121.

* not seen in original.

ISOTOPES TO ESTIMATE COLONY SIZE OF *FORMICA CINEREA* MAYR (HYMENOPTERA: FORMICIDAE)

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Abstract Ants taken from a mound were dipped in 100 ml. of water containing 0.1–0.2 millicuries of Au^{198}, I^{131} and P^{32} and then returned to the mound. After 1–3 days ants in a recapture sample were scanned singly with an end window Geiger-Muller tube to determine radioactive distintegration per minute. The colony size was estimated by the Lincoln Index. After preliminary trials, I^{131} was not used because of ant mortality, and Au^{198} because of its short half-life. A satisfactory method of using P^{32} was not developed, largely because of contamination between treated and non-treated ants, and the variable counts given by ants in the recapture sample. Experiments with dipping time, radioisotope concentrations and use of spreader-sticker additives did not materially improve the method. The variability in tagging was related to the difference in sizes of the worker ants.

Formica cinerea Mayr[2] builds conspicuous mounds on the prairies and wet meadows of southern Wisconsin. The ant mounds are the dominant feature of a prairie remnant existing along a railroad right-of-way near Platteville in southwestern Wisconsin. All stages of colony development are present, as evidenced by various sizes of mounds that range up to three feet in diameter and one foot high. The high density of colonies is indicated by a total of 160 mounds in a 1×1500 meter transect.

Studies on the ecology of *F. cinerea* have been conducted at Platteville since 1956. Quantitative population data have been obtained by digging and counting the individuals in a colony. This method is laborious, and it is uncertain whether all individuals are excavated, as some channels penetrate deeply in the well-drained soil. One objective of the research was

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[2] Det. by Dr. W. L. Brown, Jr. Wisconsin variant would be named *F. cinerea montana* Emery, according to Gregg (1953).

to determine the long-term numerical growth or decline of individual colonies. This can not be accomplished with colonies that are destroyed by digging. Therefore, there is a need to develop a reliable, indirect method of estimating the colony size.

A technique used to estimate populations is the marking method first proposed by Lincoln (1930). A certain number of animals is taken at random from a population, marked and released. A later random sample is caught and the proportion of marked animals noted. Then the total number of marked animals released divided by the proportion of marked animals in the recaptured sample is used as an estimate of the total population size. The method has been used for estimating the density of several insects, including tsetse flies (Jackson, 1940), sheep blowflies (Gilmour, Waterhouse and McIntyre, 1946), Lepidoptera (Dowdeswell, Fisher and Ford, 1949) and Odonata (Corbet, 1952). The value of the marking method is limited by certain theoretical and practical considerations of animal behavior and mortality, but the technique is simple and useful when error estimates can be made. To obtain satisfactory error estimates, Welch (1960) showed that consideration should be given to number of tagged specimens, duration of experiment, and number of recapture samples taken.

Apparently little previous work has been done on estimating the size of ant colonies by marking methods. Chew (1959) estimated the number of foraging workers in colonies of three species of ants, *Novomessor cockerelli* (Andre), *Myrmecocystus mimicus* Wheeler, and *Pogonomyrmex occidentalis* (Cresson). Ants were caught at colony entrances, etherized, and marked on the dorsum with a spot of "Testors dope." Odum and Pontin (1961) published on colony densities of *Lasius flavus* (Fab.), which were estimated on the basis of P^{32} tagging. Wide variances were shown between estimates when two recapture samples were taken subsequent to the marking. In neither of the above studies was the reliability of the method tested by actual colony counts.

Ayre (1962) conducted marking-recapture tests with the foraging workers in laboratory colonies of *Formica exsectoides* Forel, *F. fusca* (Linn.) and *Camponotus herculeanus* (Linn.). The ants were marked on the dorsum of the thorax with a "Tech-pen." The recapture sample was accomplished largely by counting all ants in a forage area and not by removing a sample of a given size. As 80 per cent of the ants did not forage under the experimental conditions, the method had little value when used to estimate the size of colonies. Even poorer results would be expected under field conditions, because temperature, humidity, state of brood, and other factors cause considerable variation in foraging activity.

We experimented with the marking-recapture method to estimate the colony size of *F. cinerea*. A satisfactory method was not developed, but

it may be of interest to report at this time on certain experiments and observations which were made in relation to testing the technique.

Method

(a) A mound of medium size, 6–8 inches in diameter and 3–5 inches high, was opened and 300 or more worker ants collected with an aspirator.
(b) The ants were tagged with a radioactive isotope by a dipping technique.
(c) The tagged ants were spread out on a piece of blotting paper placed in the mound, counted, and dead or injured ants removed. Active, apparently uninjured ants crawled off the paper into the mound.
(d) After one to three days a recapture sample was collected from the mound.
(e) Ants in the recapture sample were scanned singly for radioactive disintegration per minute, using a Tracerlab "1000" Laboratory Scaler and an end window Geiger-Müller tube.
(f) The colony size was calculated by Bailey's (1951) modification of the Lincoln Index, as follows:

$$\text{Total number of ants} = \frac{(\text{Number of ants tagged})(\text{Number of ants recaptured} + 1)}{(\text{Number of tagged ants recaptured} + 1)}$$

A dipping technique was used to tag the ants, as follows: approximately 50 ants previously aspirated into a glass vial were shaken into a 1×4.5 in. plastic vial, which was perforated with about 8 rows of 1/16 in. holes to allow easy filling and emptying of dipping fluid. The stopper was formed from a cellulose sponge. The plastic vial with the ants was dipped in 100 ml. of radioactive solution in a $1\frac{1}{4} \times 6$ in. glass cylinder. A wire handle attached to the cellulose stopper enabled the dipping tube to be lowered in the cylinder, agitated, and removed. In most experiments the ants were immersed for one minute. The dipping vial was then removed carefully to allow drainage of the excess solution back into the cylinder. The ants were shaken onto a piece of filter paper to recover from immersion. Clean plastic vials were used to dip additional ants until an adequate sample from a colony had been tagged.

Preliminary laboratory experiments were made to determine workable radioactivity levels for the isotopes Au^{198}, I^{131}, and P^{32}. Assays of 0.1–0.2 millicuries in 100 ml. of water provided a satisfactory dipping solution.

Results A field experiment with P^{32} tagging made on June 23, 1961 was inconclusive because the recapture sample was scanned for radioactivity using groups of five ants. This short cut was done on the assumption that a certain number of the lots would be negative, whereas those lots showing radioactivity could be re-scanned to determine the "hot" ant. The proce-

TABLE I. Distribution of radioactivity (counts per minute) among tagged-recaptured ants, and relation to estimate of colony density of *F. cinerea*, 1961.

Count Class	Experiment 1-Au^{198}	2-I^{131}	3-P^{32}	4-P^{32}
0	8	22	13	72
1–25	23	10	22	98
26–50	8	10	25	36
51–60	2	–	3	2
61–70	2	2	5	8
71–80	2	–	1	3
81–90	1	–	4	6
91–100	2	1	4	2
100+	6	1	23	33
Number recaptured	54	46	100	260
Number dipped	300	300	300	375
Actual density	2144	2040	1110	2369
Closest estimate	2025	1971	1111	2378
Tagged ants needed	8	7	27	41
Est. based on 100+	2700	13,800	1304	2955

Expt. 1, 2, 3, tagged July 6, recaptured July 7.
Expt. 4, tagged July 21, recaptured July 24.
All isotopes were used at 200 microcuries in 100 ml water.

dure failed because each lot showed radioactivity and it was impossible to pick out any lot having obviously tagged or non-tagged ants. For example, five ants which collectively showed 216 counts per minute gave individual counts of 14, 16, 22, 44 and 76. It was apparent that widespread contamination must exist which necessitated the scanning of each ant in a sample.

Four subsequent field trials (Table I) showed that the estimates of colony sizes varied widely in relation to the arbitrary selection of a radioactivity level which might indicate a "tagged" ant or might exclude a "contaminated" ant. This problem was analyzed by arranging the data in classes. The number of tagged ants needed to give a calculated density that approximated the actual density (as determined by digging) was selected. Counts per minute of 91+ would give the best results in treatments 1 and 3, 81+ would suffice for treatment 4, but no level would be satisfactory for treatment 2. An arbitrary selection of 100+ resulted in an overestimate in all colonies.

TABLE II. Radioactivity (counts per minute) of 10 ants dipped for one minute in various concentrations of P^{32} solution and counted 24 hours later.

Treatment	Mean	S.D.	Range
1	9,697.6	4,302.4	5,122–17,521
2	3,368.6	903.4	1,683– 4,686
3	1,763.1	1,338.9	514– 6,321
4	1,751.0	593.4	865– 3,534
5	1,024.5	771.9	238– 2,484
6	571.5	456.2	174– 1,355
7	495.8	353.2	123– 1,197
8	421.0	100.0	277– 571
9	354.8	257.2	88– 1,486

In was concluded from these results that a better method of tagging was needed to clearly distinguish dipped from contaminated ants. Therefore, laboratory tests were conducted to develop a more precise tagging method.

Ten ants were dipped, maintained in plastic petri dishes on moist filter paper, and counted singly 24 hours later. Using several concentrations of P^{32}, it was found that considerable variation existed in the counts (Table II). Using one concentration of P^{32}, no significant differences of mean radioactivity were found between ants dipped for periods of 1, 2, 4, 8, 16 and 32 minutes (Table III). The mean weights of the ants used in this

TABLE III. Radioactivity (counts per minute) and weight (milligrams) of 10 ants dipped in P^{32} solution for intervals of 1–32 minutes and counted 24 hours later.

Minutes dipped	Radioactivity Mean	S.D.	Range	Weight of ants Mean	S.D.	Range
1	728.4	340.17	226–1396	2.93	0.81	1.60–4.32
2	834.3	568.28	222–2619	1.74	2.70	.16–3.42
4	854.6	628.56	206–2713	1.94	0.87	.44–4.26
8	1083.3	490.77	197–2175	2.72	0.71	1.40–4.64
16	1047.4	457.35	272–1778	2.42	0.97	.54–4.40
32	858.0	380.43	274–1408	2.76	1.08	.96–4.68

Duncan's multiple range test: Any two treatment means not underscored by the same line are significantly different at the 1% level.

2.93(1) 2.76(32) 2.72(8) 2.42(16) 1.94(4) 1.74(2)

TABLE IV. Radioactivity (counts per minute) of 10 ants dipped for one minute in P^{32} solution or Au^{198} suspension, alone or with adhesive.

Treatment	Mean	S.D.	Range
Au^{198} alone	594.1	338.2	343–1183
Au^{198} with glue	310.2	141.2	150– 639
Au^{198} alone	5893.1	2041.4	2546–9161
Au^{198} with glue	6018.3	1630.9	3756–8405
P^{32} alone	606.5	323.3	304–1177
P^{32} with glue	740.5	317.3	364–1230
P^{32} alone	946.6	601.5	453–2815
P^{32} with glue	1055.4	313.1	616–1481

experiment varied significantly at the 1% level. Duncan's multiple range test showed that the means of treatments for 2 and 4 minutes were significantly different from the other treatments and from each other. The addition of 1 ml. of "Elmers" glue (Borden) to 100 ml. of isotope solution did not significantly increase the radioactivity counts, and did not reduce the wide variation between the counts (Table IV).

Various combinations of dipping time, spreader-sticker (DuPont) and concentrations of P^{32} were tested. The 150 ml. dipping solutions included X and 2X concentrations of P^{32}, with or without 1 ml. of sticker solution. The stock solution of spreader-sticker contained 3 drops in 500 ml. of water.

The X concentration of P^{32} used in experiment A was 0.125 millicuries; in B 0.137. In A, 20 ants were dipped on August 7 and counted on August 9, 1962. In B, 20 ants were dipped on August 17, first counted on August 20 (B_1), then held on moist filter paper in plastic petri dishes, and recounted on August 29 (B_2). The results of the treatments analyzed by Duncan's multiple range test at the 5% level are given in Table V.

The data obtained from experiment A showed that ants dipped ten minutes in the 2X concentration of P^{32} gave the highest counts per minute of radioactivity, but that the addition of a spreader-sticker did not significantly increase counts. In B, the ten minute dip and 2X concentration was sig-

TABLE V. Radioactivity (counts per minute) of 20 ants treated with various combinations of dipping time, spread-sticker and concentrations of P^{32}.

Treatment	Minutes dipped	Spreader-sticker	P^{32} concentration
1	10	+	2 X
2	10	−	2 X
3	10	+	X
4	10	−	X
5	5	+	2 X
6	5	−	2 X
7	5	+	X
8	5	−	X

Duncan's multiple range test: Any two treatment means not underscored by the same line are significantly different at the 5% level.

Experiment A

Treatment	2	1	6	4	5	3	7	8
Mean	704.1	617.7	519.9	402.3	402.3	340.9	329.7	194.5

Experiment B_1

Treatment	1	4	5	3	7	6	2	8
Mean	485.4	275.3	263.7	202.9	169.6	160.8	134.2	80.0

Experiment B_2

Treatment	1	5	3	6	2	7	8	4
Mean	222.2	214.1	130.3	116.5	116.3	103.4	63.9	61.1

nificantly better at 3 days, but at 12 days was not significantly better than ants dipped for 5 minutes. It will be noted that the arrangement of the means differed considerably at 5 and 12 days. It was concluded that neither longer dipping times, addition of spreader-sticker, nor increased concentration of the P^{32} solution could be depended upon to give a consistent significant increase in the radioactivity counts.

In the above experiments, untreated check ants were maintained on moist filter paper in the plastic dishes under conditions similar to the tagged ants. Dipping of ants in various solutions of P^{32} did not cause mortality greater than normally experienced in the checks. It should be mentioned that dipping in I^{131} solutions caused considerable mortality and this isotope was

discarded after the preliminary trials. Also, Au^{198} was not tested extensively because the short half-life of the isotope (2.7 days) makes it unsuitable for use in a scheme of sequential sampling.

Discussion The accuracy of the marking-recapture method is determined to a large extent by the validity of assumptions that a random sample is taken from the population, the individuals are not injured, adverse behavior does not occur, and re-mixing is thorough. An estimate of the magnitude of error can be calculated by several methods (Bailey, 1951; Welch, 1960). Usually, a series of recaptures is made. The precision of the sequential sampling depends upon a "permanent" marking technique, a knowledge of the death- and birth-rates during the intervening periods, and a measure of the migration habits of the population.

We could not conduct detailed studies on any of the above assumptions underlying the use of the marking-recapture method because we did not develop the first requisite, namely a suitable method of marking the ants. There was no problem tagging the ants with radioisotopes, but the widespread contamination between the tagged and non-tagged ants made identification of the primary tag difficult. Some contamination was expected because of the well-known mechanism of food transmission (trophallaxis) among ants. Wilson and Eisner (1957) fed a mixture of honey and radioactive iodine to a single worker ant, and traced the spread of radioactivity throughout laboratory colonies of five species, including *Formica* spp. Kannowski (1959) fed ants a mixture of honey and radioactive phosphorus to study colony distribution of *Lasius minutus* Emery. Both investigations showed that food is passed from worker to worker in a relatively short time in *Lasius* and *Formica* spp.

Laboratory experiments with dipping time, radioisotope concentrations, and use of spreader-sticker additives, did not materially improve the method. No technique was found that significantly reduced the wide variation in levels of radioactivity among the tagged ants. This variability, along with the widespread contamination, made it impossible to pick out with certainty the tagged ants in a recaptured sample.

It is believed that the variability in tagging was related to the difference in sizes of worker ants. Ten random samples of 25 ants each from field and laboratory colonies were weighed, and each sample showed considerable variation. The weights of ants ranged from 0.16 to 5.02 milligrams. However, a correlation analysis on the radioactivity vs. weights of 21 ants selected at random from the recaptured ants in experiment 4, table I, gave a non-significant correlation coefficient of 0.205.

The effect of differences in foraging behavior and sampling locations was discussed by Ayre (1962). Samples taken at the nest opening or in the immediate vicinity of the nest would contain a disproportion of workers

that are engaged in construction, cleaning or repairing the nest. In our experiments we questioned whether the ant sample taken from the mound adequately represented the colony, or a suitable remixture occurred in the interval between marking and recapture. In experiment 1, table I, twenty-one additional ants were obtained from the underground cavity of the colony, and scanned for radioactivity. Six ants had a zero count, and 15 were in the 1–25 counts per minute class. This low level of contamination suggested that some intermixture took place between ants in the mound and ants in the cavity. The percentage of worker ants in the mound changes considerably during the season. Normally only a few ants are present in the mound when brood is absent. Population data from 35 colonies (unpublished) gave an average of 14.4 per cent of the workers in the mound (range 66.3–0.5%). If little intermixture occurs between mound and cavity ants, or if there is a division of labor among the ants, then it is possible that a mound sample may not give a valid representation of the total colony size of *F. cinerea.*

The lack of a suitable marking technique has applied to other ant research. Chew (1959) reported that colored dope flaked off in a few days. His re-sampling data indicated a gradual increase in colony size, which could indicate that the marked individuals were losing their identification. Ayre (1962) found the usefulness of his marking method limited to ten days. Reciprocal cleaning activities of the ants resulted in the complete removal of ink in 2–3 months. Odum and Pontin (1961) reported no problem, as in all cases it was easily possible on the basis of radioactivity to distinguish the ants which had been soaked in P^{32} and those which had not. They stated that in some cases untagged ants picked up a small amount of radioactivity, but the difference between "primary" and "secondary" tagging was so great that there was never any doubt as to which individual had received the primary tag. In their experiment the tagged ants were determined with a scanning system which automatically recorded the presence of radioactive ants, but apparently did not indicate the levels of radioactivity.

A suitable radioactive marking method should offer considerable promise from the standpoint of a general theory of population sampling in ants. In a large population, it is difficult and laborious to mark enough specimens singly by a hand method to obtain a sufficient number of recaptures. The dipping of ants in a radioactive material would seem to be worth developing, especially if the optimum number of specimens to be marked can be estimated by a prior knowledge of ant biology and relations between mound size and colony density.

Literature cited

Ayre, G. L. 1962. Problems in using the Lincoln Index for esitmating the size of ant colonies. Jour. N. Y. Ent. Soc. **70**: 159–166.

Bailey, N. T. J. 1951. On estimating the size of mobile populations from recapture data. Biometrika **38**: 293–306.

Chew, R. M. 1959. Estimation of ant colony size by the Lincoln Index method. Jour. N. Y. Ent. Soc. **67**: 157–161.

Corbet, P. S. 1952. An adult population study of *Pyrrhosoma nymphula* (Sulzer). Jour. Anim. Ecol. **21**: 206–222.

Dowdeswell, W. H., Fisher, R. A. and Ford, E. B. 1949. The quantitative study of populations in the Lepidoptera. Heredity **3**: 67–84.

Gilmour, D., Waterhouse, D. F. and McIntyre, G. A. 1946. An account of experiments undertaken to determine the natural population density of the sheep blowfly, *Lucilia cuprina* Wied. Australia Council Sci. & Ind. Res. Bul. **195**, 39 pp.

Gregg, R. E. 1953. Morphological considerations affecting the taxonomy of certain genera of ants. Proc. Ent. Soc. Wash. **55**: 324–330.

Jackson, C. H. N. 1940. The analysis of tsetse fly population. Ann. Eugen. London **10**: 332–369.

Kannowski, P. B. 1959. The use of radioactive phosphorous in the study of colony distribution of the ant *Lasius minutus*. Ecology **40**: 162–165.

Lincoln, F. C. 1930. Calculating waterfowl abundance on the basis of banding returns. U. S. Dept. Agric. Circ. 118, 4 pp.

Odum, E. P. and Pontin, A. J. 1961. Population density of the underground ant, *Lasius flavus*, as determined by tagging with P^{32}. Ecology **42**: 186–188.

Welch, H. E. 1960. Two applications of a method of determining the error of population estimates of mosquito larvae by the mark and recapture method. Ecology **41**: 228–229.

Wilson, E. O. and Eisner, T. 1957. Quantitative studies of liquid food transmission in ants. Ins. Sociaux **4**: 157–166.

COMPOSITION OF EXUVIAE OF THE MEALWORM, TENEBRIO MOLITOR LINNAEUS

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Abstract Prepupal exuviae weigh 2.19, while those of the pupa 1.09 mg. Values for water content are 11.35 and 17.18%, respectively. In each case these means are statistically different. Nitrogen is approximately 10.7% of dry weight in each case. Lipid N (Fraction A), expressed as % total N, is 0.75 in prepupal and 0.66 in pupal exuviae. Water soluble N fractions (B soluble, and C insoluble in tungstic acid) are significantly lower in prepupal than in pupal exuviae. Values for B are 8.06 and 15.65; and for C, 1.19 and 2.92, respectively. Those for insoluble N (Fraction D) are 89.95 and 80.78, respectively.

During observations on the effects of parental age on the development of the mealworm, *Tenebrio molitor*, (Ludwig and Fiore, 1960) exuviae of prepupae and pupae were obtained in large numbers. Since the exuviae of the two stages are very different in appearance, it was decided to determine